

WHAT IS CLAIMED IS:

- 5 1. A method of detecting or quantifying a target nucleic acid having a predetermined sequence in a specimen comprising:
- (a) preparing a probe A and a probe B,  
said probe A being a first probe which has a sequence F' complementary to a first partial sequence F of the target nucleic acid and a binding molecule bound to the sequence F', and
- 10 said probe B being a second probe which has a sequence S' complementary to a second partial sequence S of the target nucleic acid and a flag bound to the sequence S', where said flag is a double-stranded sequence and has a marker substance in one of the
- 15 double strand;
- (b) hybridizing the first probe A with the first partial sequence F of the target nucleic acid and hybridizing the second probe B with the second partial sequence S of the target nucleic acid;
- 20 (c) ligating the first probe A and the second probe B both being hybridized with the target nucleic acid, thereby obtaining a probe (A+B);
- (d) binding the binding molecule to a substance capable of being paired up therewith, thereby
- 25 recovering the probe (A+B); and
- (e) recovering a single-stranded nucleic acid having the marker substance of the double stranded

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2. A method of detecting or quantifying target nucleic acids N1-Nn (n is an integer of 2 or more), each having a predetermined sequence, in a specimen, comprising:

said probes A1-An being first probes which respectively have sequences F1'-Fn' (n is an integer of 2 or more) complementary to first partial sequences F1-Fn (n is an integer of 2 or more) of the target nucleic acids and a binding molecule bound to each of the sequences F1'-Fn', and

(b) respectively hybridizing the first probes A1-An with the first partial sequences F1-Fn of the target nucleic acids, and simultaneously hybridizing

the second probes B1-Bn with the second partial sequences S1-Sn of the target nucleic acids, respectively;

5 (c) respectively ligating the first probes A1-An and the second probes B1-Bn, both being hybridized with the target nucleic acids, respectively, thereby obtaining probes (A1+B1)-(An+Bn) (n is an integer of 2 or more);

10 (d) binding the binding molecule to a substance capable of being paired up therewith, thereby recovering the probes (A1+B1)-(An+Bn); and

15 (e) recovering a single-stranded nucleic acid having the marker substance from the double-stranded nucleic acid constituting each of the flags and detecting or quantifying the marker substance, thereby detecting or quantifying each of the target nucleic acids N1-Nn in the specimen.

20 3. A method of detecting or quantifying a target nucleic acid having a predetermined sequence, in a specimen, comprising:

25 (a) preparing a probe A and a probe B, said probe A being a first probe which has a sequence F' complementary to a first partial sequence F of the target nucleic acid and a tag sequence Tg bound to the sequence F', and

said probe B being a second probe which has a sequence S' complementary to a second partial sequence

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S of the target nucleic acid and a marker substance bound to the sequence S'

5 (b) mixing the probe A, the probe B, and the specimen, thereby hybridizing the probe A with the first partial sequence F of the target nucleic acid and simultaneously hybridizing the probe B with the second partial sequence S of the target nucleic acid;

10 (c) ligating the probe A and the probe B, both being hybridized with the target nucleic acid, thereby obtaining a probe (A+B);

(d) dissociating the probe (A+B) from the target nucleic acid;

15 (e) hybridizing the tag sequence Tg with a sequence Tg' complementary to the tag sequence Tg, thereby recovering the probe (A+B); and

(f) detecting or quantifying the marker substance in the probe (A+B) recovered, thereby detecting or quantifying the target nucleic acid in the specimen.

20 4. A method of detecting or quantifying nucleic acids N1-Nn, each having a predetermined sequence, in a specimen, comprising:

(a) preparing probes A1-An (n is an integer of 2 or more) and probes B1-Bn (n is an integer of 2 or more),

25 said probes A1-An being first probes which respectively have sequences F1'-Fn' (n is an integer of 2 or more) complementary to first partial sequences

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F1-Fn (n is an integer of 2 or more) of the target nucleic acids N1-Nn (n is an integer of 2 or more) and tag sequences Tg1-Tgn bound to the sequences F1'-Fn', and

5           said probes B1-Bn being second probes which respectively have sequences S1'-Sn' (n is an integer of 2 or more) complementary to second partial sequences S1-Sn (n is an integer of 2 or more) of the target nucleic acids N1-Nn and the marker substance bound to  
10           each of the sequences S1'-Sn' of the target nucleic acid;

          (b) mixing the probes A1-An, the probes B1-Bn, and the specimen, thereby hybridizing probes A1-An respectively with the first partial sequences F1-Fn of  
15           the target nucleic acids N1-Nn, and simultaneously hybridizing the probes B1-Bn with the second partial sequences S1-Sn of the target nucleic acids N1-Nn, respectively;

          (c) respectively ligating probes A1-An and the  
20           probes B1-Bn, both being hybridized with the target nucleic acids, respectively, thereby obtaining probes (A1+B1)-(An+Bn) (n is an integer of 2 or more);

          (d) dissociating the probes (A1+B1)-(An+Bn) from the target nucleic acids;

25           (e) hybridizing sequences Tg1-Tgn respectively with sequences Tg1'-Tgn' complementary to the tag sequences Tg1-Tgn, thereby recovering the probes

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(A1+B1)-(An+Bn); and

(f) detecting or quantifying the marker substance in the probes (A1+B1)-(An+Bn) recovered, thereby detecting or quantifying the target nucleic acids N1-Nn in the specimen.

5           5. A method of detecting or quantifying a target nucleic acid having a predetermined sequence in a specimen, comprising:

          (a) preparing a probe A and a probe B,

10           said probe A being a first probe which has a sequence F' complementary to a first partial sequence F of the target nucleic acid and a tag sequence Tg bound to the sequence F', and

          said probe B being a second probe which has a  
15           sequence S' complementary to a second partial sequence S of the target nucleic acid, a flag sequence FL bound to the sequence S', and a marker substance bound to the flag sequence FL;

20           (b) mixing the probe A, the probe B, and the specimen, thereby hybridizing the probe A with the first partial sequence F of the target nucleic acid and simultaneously hybridizing the probe B with the second partial sequence S of the target nucleic acid;

25           (c) ligating the probe A and the probe B, both being hybridized with the target nucleic acid, thereby obtaining a probe (A+B);

          (d) dissociating the probe (A+B) from the target

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nucleic acid;

(e) hybridizing the tag sequence Tg contained in the probe (A+B) with a sequence Tg' complementary to the tag sequence Tg, thereby dissociating the probe (A+B); and

(f) recovering a portion containing at least the probe B from the probe (A+B) hybridized with the sequence Tg';

(g) hybridizing the flag sequence FL recovered with a nucleic acid sequence FL' complementary to the flag sequence FL, thereby specifically recovering the portion containing at least probe B; and

(h) selectively detecting the marker substance contained in the portion containing at least the probe B recovered, thereby detecting or quantifying the target nucleic acid in the specimen.

6. A method of detecting or quantifying nucleic acids N1-Nn (n is an integer of 2 or more), each having a predetermined sequence, in a specimen, comprising:

(a) preparing probes A1-An (n is an integer of 2 or more) and probes B1-Bn (n is an integer of 2 or more),

said probes A1-An being first probes which respectively have sequences F1'-Fn' (n is an integer of 2 or more) complementary to first partial sequences F1-Fn of the target nucleic acids N1-Nn (n is an integer of 2 or more), respectively, and tag sequences

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Tg1-Tgn bound to the sequences F1'-Fn', respectively,  
and

said probes B1-Bn being second probes which  
respectively have sequences S1'-Sn' (n is an integer of  
2 or more) complementary to second partial sequences  
S1-Sn (n is an integer of 2 or more) of the target  
nucleic acids N1-Nn, flag sequences FL1-FLn bound to  
the sequences S1'-Sn', and a marker substance bound to  
each of the flag sequences FL1'-FLn';

(b) mixing the probes A1-An, the probes B1-Bn,  
and the specimen, hybridizing probes A1-An respectively  
with the first partial sequences F1-Fn of the target  
nucleic acids N1-Nn, and simultaneously hybridizing the  
probes B1-Bn with the second partial sequences S1-Sn of  
the target nucleic acids N1-Nn, respectively;

(c) respectively ligating probes A1-An and second  
probes B1-Bn, both being hybridized with the target  
nucleic acids, thereby obtaining probes (A1+B1)-(An+Bn)  
(n is an integer of 2 or more);

(d) dissociating the probes (A1+B1)-(An+Bn) from  
the target nucleic acids;

(e) hybridizing tag sequences Tg1-Tgn contained  
in the probes (A1+B1)-(An+Bn) with sequences Tg1'-Tgn'  
complementary to the tag sequences Tg1-Tgn, thereby  
dissociating the probes (A1+B1)-(An+Bn); and

(f) recovering portions respectively containing  
at least the probes B1-Bn, from the probes

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(A1+B1)-(An+Bn) hybridized with the sequence Tg1'-Tgn';

(g) hybridizing the flag sequences FL1-FLn with nucleic acid sequences FL1'-FLn' complementary to the flag sequences FL1-FLn, thereby specifically recovering the portions respectively containing at least probes B1-Bn; and

(h) selectively detecting the marker substance contained in the portions respectively containing at least the probes B1-Bn recovered, thereby detecting or quantifying the target nucleic acids N1-Nn in the specimen.

7. A method of detecting or quantifying nucleic acids N1-Nn (n is an integer of 2 or more), each having a predetermined sequence, in a specimen, comprising:

(a) preparing probes A1-An (n is an integer of 2 or more) and probes B1-Bn (n is an integer of 2 or more),

said probes A1-An being first probes which respectively have sequences F1'-Fn' (n is an integer of 2 or more) complementary to first partial sequences F1-Fn of the target nucleic acids N1-Nn (n is an integer of 2 or more) and tag sequences Tg1-Tgn bound to the sequences F1'-Fn', respectively, and

said probes B1-Bn being second probes which respectively have sequences S1'-Sn' (n is an integer of 2 or more) complementary to second partial sequences S1-Sn (n is an integer of 2 or more) of the target

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nucleic acids N1-Nn, and a marker substance bound to each of the sequences S1'-Sn'

(b) mixing the probes A1-An, the probes B1-Bn, and the specimen, thereby hybridizing probes A1-An respectively with the first partial sequences F1-Fn of the target nucleic acids N1-Nn, and simultaneously hybridizing the probes B1-Bn with the second partial sequences S1-Sn of the target nucleic acids N1-Nn, respectively;

(c) respectively ligating probes A1-An and second probes B1-Bn, both being hybridized with the target nucleic acids N1-Nn, thereby obtaining probes (A1+B1)-(An+Bn) (n is an integer of 2 or more);

(d) hybridizing tag sequences Tg1-Tgn with sequences Tg1'-Tgn' complementary to the tag sequences Tg1-Tgn, thereby recovering the probes (A1+B1)-(An+Bn); and

(e) detecting or quantifying the marker substance contained in the probes (A1+B1)-(An+Bn) recovered, thereby detecting or quantifying the target nucleic acids N1-Nn in the specimen,

wherein Tm values of the tag sequences Tg1-Tgn are higher than Tm values of sequences F1-Fn and sequences S1-Sn.

8. A method of detecting or quantifying a target nucleic acid having a predetermined sequence in a specimen comprising:

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(a) preparing a probe A and a probe B,  
said probe A being a first probe which has a  
sequence F' complementary to a first partial sequence F  
of the target nucleic acid and a binding molecule bound  
5 to the sequence F', and

said probe B being a second probe which has a  
sequence S' complementary to a second partial sequence  
S of the target nucleic acid and a flag sequence FL  
consisting of 4 units bound to the sequence S', where  
10 said flag FL sequence hybridizes with a sequence FL'  
bound to the sequence S' to form a double-stranded  
sequence; and

(b) mixing the probe A, probe B and the specimen,  
thereby hybridizing the probe A with the first partial  
15 sequence F of the target nucleic acid, and  
simultaneously hybridizing the second probe B with the  
second partial sequence S of the target nucleic acid;

(c) ligating the probe A and the probe B, both  
being hybridized with the target nucleic acid, thereby  
20 obtaining a probe (A+B);

(d) binding the binding molecule to a substance  
capable of being paired up therewith, thereby  
recovering the probe (A+B); and

(e) denaturing the double-stranded flag sequence  
25 of the probes (A+B) recovered into single-stranded flag  
sequence;

(f) hybridizing the single-stranded flag sequence

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with two primers one of which has a binding molecule B and the other of which has a marker substance L, and extending the primers to form a complementary strand of the flag sequence FL, thereby obtaining a double strand;

(g) binding a binding molecule B with a substance capable of being paired with the binding molecule B, thereby recovering the double strand; and

(h) detecting or quantifying the target substance L, thereby detecting or quantifying the target nucleic acid in the specimen.

9. A method of detecting or quantifying target nucleic acids N1-Nn (n is an integer of 2 or more), each having a predetermined sequence, in a specimen, comprising:

(a) preparing probes A1-An (n is an integer of 2 or more) and probes B1-Bn (n is an integer of 2 or more),

said probes A1-An being first probes which respectively have sequences F1'-Fn' (n is an integer of 2 or more) complementary to first partial sequences F1-Fn (n is an integer of 2 or more) of the target nucleic acids and a binding molecule bound to each of the sequences F1'-Fn', and

said probes B1-Bn (n is an integer of 2 or more) being second probes which respectively have sequences S1'-Sn' (n is an integer of 2 or more) complementary to

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second partial sequences S1-Sn (n is an integer of 2 or more) of the target nucleic acids, and flag sequences FL1-FLn each consisting of 4 units, bound to the sequences S1'-Sn', where said flag sequences FL1-FLn hybridize respectively with sequences FL1'-FLn' bound to the sequences S1'-Sn' to form double-stranded sequences; and

(b) mixing the probes A1-An, the probes B1-Bn, and the specimen, thereby hybridizing probes A1-An respectively with the first partial sequences F1-Fn of the target nucleic acids N1-Nn, and simultaneously hybridizing the probes B1-Bn with the second partial sequences S1-Sn of the target nucleic acids N1-Nn;

(c) respectively ligating the probes A1-An and the probes B1-Bn, both being hybridized with the target nucleic acids N1-Nn, thereby obtaining probes (A1+B1)-(An+Bn);

(d) binding each of the binding molecules to a substance capable of being paired up therewith, thereby recovering the probes (A1+B1)-(An+Bn); and

(e) denaturing double-stranded flag sequences of the probes (A+B)-(An+Bn) recovered into single-stranded flag sequences;

(f) hybridizing the single-stranded flag sequences FL1-FLn with two primers one of which has a binding molecule B and the other of which has a marker substance L, and extending the two primers, to form

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complementary strands of the flag sequences FL1-FLn,  
thereby obtaining double strands;

(g) binding a binding molecule B with a substance  
capable of being paired therewith, thereby recovering  
5 the double strands; and

(h) detecting or quantifying the marker substance  
L, thereby detecting or quantifying the target nucleic  
acids N1-Nn in the specimen.

10. A method of detecting or quantifying a target  
10 nucleic acid having a predetermined sequence in a  
specimen comprising:

(a) preparing a probe A and a probe B,

15 said probe A being a first probe which has a  
sequence F' complementary to a first partial sequence F  
of the target nucleic acid and a binding molecule bound  
to the sequence F', and

20 said probe B being a second probe which has a  
sequence S' complementary to a second partial sequence  
S of the target nucleic acid and a flag consisting of 4  
units bound to the sequence S', where said flag FL is a  
double-stranded sequence; and

25 (b) mixing the probe A, the probe B, and the  
specimen, thereby hybridizing the probe A with the  
first partial sequence F of the target nucleic acid and  
simultaneously hybridizing the probe B with the second  
partial sequence S of the target nucleic acid;

(c) ligating the probe A and the probe B, both

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being hybridized with the target nucleic acid, thereby obtaining a probe (A+B);

(d) binding the binding molecule to a substance capable of being paired up therewith, thereby recovering the probe (A+B); and

(e) denaturing the double-stranded nucleic acid constituting the flag into single-stranded nucleic acid;

(f) amplifying the single-stranded nucleic acid present in a liquid phase by PCR, thereby performing an encode reaction;

(g) performing transcription of a sequence FL' complementary to the single stranded flag sequence obtained by the encode reaction, by use of two primers one of which is a primer having another binding molecule and the other of which is a primer having a marker substance, thereby performing a decode reaction;

(h) binding said another binding molecule to a substance being paired up therewith, recovering a nucleic acid molecule obtained by the decode reaction; and

(i) detecting or quantifying the marker substance, thereby detecting or quantifying the target nucleic acid.

11. A method of detecting or quantifying target nucleic acids N1-Nn (n is an integer of 2 or more), each having a predetermined sequence, in a specimen,

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(A1+B1)-(An+Bn) (n is an integer of 2 or more);

(d) binding the binding molecule to a substance capable of being paired up therewith, to recover the probes (A1+B1)-(An+Bn), and thereafter performing an encode reaction of each of the flags FL1-FLn; and

(e) performing a decode reaction of the sequences FL1'-FLn' complementary to the flags FL1-FLn obtained by the encode reaction; and

(f) detecting or quantifying the nucleic acid molecules obtained by the decode reaction, thereby detecting or quantifying the target nucleic acids N1-Nn in the specimen.

12. A method of detecting or quantifying target nucleic acids N1-Nn (n is an integer of 2 or more), each having a predetermined sequence, in a specimen, comprising:

(a) preparing probes A1-An (n is an integer of 2 or more) and probes B1-Bn (n is an integer of 2 or more),

said probes A1-An being first probes which respectively have sequences F1'-Fn' (n is an integer of 2 or more) complementary to first partial sequences F1-Fn (n is an integer of 2 or more) of the target nucleic acids and a binding molecule bound to each of the sequences F1'-Fn', and

said probes B1-Bn (n is an integer of 2 or more) being second probes which respectively have sequences

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S1'-Sn' (n is an integer of 2 or more) complementary to second partial sequences S1-Sn (n is an integer of 2 or more) of the target nucleic acids and flag sequences FL1-FLn each consisting of 4 units, bound to the sequences S1'-Sn', respectively,

(b) mixing the probes A1-An, the probes B1-Bn, and the specimen, thereby hybridizing probes A1-An respectively with the first partial sequences F1-Fn of the target nucleic acids N1-Nn, and simultaneously hybridizing the probes B1-Bn with the second partial sequences S1-Sn of the target nucleic acids N1-Nn, respectively;

(c) respectively ligating the probes A1-An and the probes B1-Bn, both being hybridized with the target nucleic acids N1-Nn, thereby obtaining probes (A1+B1)-(An+Bn);

(d) binding each of the binding molecules to a substance capable of being paired up therewith to recover the probes (A1+B1)-(An+Bn), and thereafter performing an encode reaction for each of the flags FL1-FLn; and

(e) performing a decode reaction of the sequences F11'-FLn' complementary to the flags FL1-FLn (n is an integer of 2 or more) obtained by the encode reaction; and

(h) detecting the nucleic acid molecules obtained by the decode reaction, thereby detecting or

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quantifying the target nucleic acids N1-Nn in the specimen,

wherein 2 units of 4 units are sequences functioning as primers for PCR amplification.

5        13. A method of detecting or quantifying a target nucleic acid having a predetermined sequence in a specimen comprising:

(a) preparing a probe A and a probe B,

10        said probe A being a first probe which has a sequence F' complementary to a first partial sequence F of the target nucleic acid and a binding molecule bound to the sequence F', and

15        said probe B being a second probe which has a sequence S' complementary to a second partial sequence S of the target nucleic acid and a flag consisting of 4 units bound to the sequence S', where said flag FL is a double-stranded sequence and said 4 units consist of SD, D0, D1 and ED each having an arbitrary sequence, bounded to each other sequentially in the order  
20        mentioned; and

(b) mixing the probe A, the probe B, and the specimen, thereby hybridizing the probe A with the first partial sequence F of the target nucleic acid and simultaneously hybridizing the probe B with the second  
25        partial sequence S of the target nucleic acid;

(c) ligating the probe A and the probe B both being hybridized with the target nucleic acid, thereby

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obtaining a probe (A+B);

(d) binding the binding molecule to a substance capable of being paired up therewith, thereby recovering the probe (A+B); and

5 (e) denaturing the double-stranded nucleic acid constituting the flag into a single-stranded nucleic acid;

(f) hybridizing the single-stranded nucleic acid obtained in a liquid phase with sequences complementary to sequences D11-D1n labeled with a marker substance, as primers,

(g) extending the primers hybridized

(h) denaturing a double-stranded nucleic acid having primers into a single-stranded nucleic acid;

15 (i) hybridizing the sequences D01-D0n specifically with the primers extended to detect or quantify the marker substances included in the sequences D01-D0n, thereby detecting or quantifying the target nucleic acids.

20 14. The method according to claims 10 to 12, wherein the decode reaction comprises, where said flag(s) FL is a double-stranded sequence and said 4 units consist of SD, D0, D1 and ED each having an arbitrary sequence, bound to each other sequentially in the order mentioned,

25 (i) performing PCR for a single-stranded sequence encoded using SD sequence to which a binding molecule

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is attached, and ED sequence, as primers;

(ii) binding a binding molecule bound to the SD sequence to a substance capable of being paired up therewith, thereby recovering a PCR product;

5 (iii) denaturing the PCR produce into a single strand

(iv) hybridizing the single strand with primers D11'-D1n' labeled;

(v) extending the primers;

10 (vi) denaturing the primers extended into single strands;

(vii) hybridizing extended single strands of the primers with sequences D01-D0n to detect or quantify marker substances included in that sequences D01-D0n, thereby detecting or quantifying the target nucleic acid.

15 15. The method according to claims 10 to 12, wherein the decode reaction comprises, where said flag FL is a double-stranded sequence and said 4 units consist of SD, D0, D1 and ED each having an arbitrary sequence, bound to each other sequentially in the order mentioned; and

20 (i) performing PCR for a single-stranded sequence encoded using SD sequence to which a binding molecule is attached and ED sequence, as primers;

(ii) binding the binding molecule bound to the SD sequence to a substance capable of being paired up

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therewith, thereby recovering a PCR product;

(iii) denaturing the PCR product into a single strand;

5 (iv) mixing the sequences D1n' labeled and D0n' labeled, thereby hybridizing the single strand with the sequences D1n' and D0n';

(v) ligating the sequence D1n' with the sequence D0n';

10 (vi) denaturing the sequences ligated into a single-stranded sequence;

(vii) hybridizing sequences D01-D0n with the single-stranded sequence labeled with a marker substance, to detect or quantify the marker substance, thereby detecting or quantifying the target nucleic acid.

15 16. The method according to any one of claims 1 to 13, wherein said first partial sequence and said second partial sequence are positioned next to each other.

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